

Vaccines, cells and nucleic acids*

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Introduction

The use of continuous cell lines as substrates for propagation of viruses and for growth of recombinant vectors in order to produce antigens for use as vaccines has been the subject of protracted debate, primarily from the point of view of vaccine safety and particularly the possibility of induction of transformation and frank neoplastic changes in the recipient (1).

Progress in basic biology and in recombinant DNA technology is providing the means for the manufacture of biological products on a larger scale than ever before. Thus, the potential usefulness of continuous cell lines as substrates has become immediately apparent. In 1981, the WHO Expert Com-

mittee on Biological Standardization (2) approved the use of non-tumorigenic and virus-free continuous cell lines for the production of killed poliomyelitis virus vaccine. A major concern was the long-term risk of malignant changes posed by (i) heterogeneous contaminating DNA, especially if the DNA were to contain potentially oncogenic coding or regulatory sequences either from the host cell or when used in the construction of recombinant vectors, (ii) the use of cells containing endogenous viruses or persisting complete or incomplete viral genomes, and (iii) the biological effects of transforming proteins. After much discussion, particular emphasis was placed on achieving safety of biological products at all stages by:

- the use of normal cell substrates and ensuring freedom from contamination by adventitious agents;
- the extensive characterization of the cell substrate and rigorous testing of the final products to ensure freedom from detectable antigenic contaminants, adventitious agents (including retroviruses) and their products, and residual host-cell DNA.

A WHO Study Group, which met in 1986 (3), concluded that, in general, continuous mammalian cell lines are acceptable as substrates for the production of biologicals, but that differences in the nature of the products and in the characteristics of the manufacturing process must be taken into account in making a decision on the acceptability of a given product (4).

A meeting of a group of experts at the Royal Free Hospital School of Medicine, London, on 17–19 July 1989 reviewed the theoretical and available experimental information in relation to the safety and possible hazards from the use of vaccines for prophylaxis of millions of healthy individuals and infants, but not from therapeutic biological substances employed for the treatment of patients. The participants considered the safety of recombinant vaccines produced in bacteria, yeast and other eukaryotic cells, including avian, insect and mammalian cells, as well as the use of various vector constructs in such cells to produce the desired antigens. Their principal concern was the safety of such materials, which lies principally with residual DNA in the vaccine and also with gene

*This report of a Workshop on Vaccines prepared in Continuous Mammalian Cell Lines, which was held in London, England, on 17–19 July 1989, was prepared by the authors on behalf of the following participants: T. Bektimirov and D. Magrath (WHO, Geneva, Switzerland); J. Coffin (Tufts University, Boston, MA, USA); F. Deinhardt (Max v. Pettenkofer Institute, Munich, Federal Republic of Germany); G. Eder (Immuno AG, Vienna, Austria); R. Ellis (Merck Sharp & Dohme Research Laboratories, West Point, PA, USA); E. Esber (Food and Drug Administration, Bethesda, MD, USA); D.A. Galloway (Fred Hutchinson Cancer Research Centre, Seattle, WA, USA); T. Harrison and A.J. Zuckerman (Royal Free Hospital School of Medicine, London, England); M. Hilleman (Merck Institute for Therapeutic Research, West Point, PA, USA); P. Howley (National Cancer Institute, Bethesda, MD, USA); P. Hofschneider (Max Planck Institute, Martinsreid, Federal Republic of Germany); E. Kieff (Harvard School of Medicine, Boston, MA, USA); L. Lasky (Genentech Inc, South San Francisco, CA, USA); J. Lower (Paul Ehrlich Institute, Frankfurt, Federal Republic of Germany); P. Minor and G. Schild (National Institute for Biological Standardisation and Control, Potters Bar, England); J. Peetermans (Smith Kline Biologicals, NV, Belgium); W. Robinson (Stanford University School of Medicine, Stanford, CA, USA); and H. Temin (University of Wisconsin, Madison, WI, USA). A French translation of this article will appear in a later issue of the *Bulletin*.

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products such as regulatory proteins. The issues discussed are described below.

Continuous cell lines

DNA from cell systems

DNA from the cell system, including components of the culture medium and all manufacturing processes, used for production of the vaccine can be derived from DNA of cell genomes as well as cells and nucleic acids from adventitious agents.

Out of the many possible effects on cells of the integration of heterogeneous DNA, the only one considered to be of importance is the induction of premalignant changes as a result of activation of proto-oncogenes, insertion of oncogenes, or inactivation of tumour suppressor genes. Other effects such as killing of individual cells, or inducing their dysfunction, for example by gene inactivation, were thought to be relatively unimportant.

Heterogeneous DNA is defined here as a complex set of sequences such as cellular DNA of high complexity in contrast to DNA of limited complexity such as viral genomes or molecularly cloned sequences. The three possible biological effects from the integration of contaminating heterogeneous DNA are:

- Introduction of activated proto-oncogenes which are present in significant amounts only in neoplastic or preneoplastic cells.
- Activation of proto-oncogenes, which is more likely to occur with mammalian DNA but is also possible with any DNA.
- Inactivation of tumour suppressor genes which can be caused by any DNA.

Other side-effects from the use of nucleic acids derived from cell culture systems in vaccine production could be due to exposure to infectious nucleic acids of agents pathogenic to man, including possibly an infectious form of a spongiform encephalopathy agent which may or may not be nucleic acid.

Cell systems containing an infectious contaminating virus should never be used in vaccine production.

DNA from the vector constructs used for expression of antigens

These vectors may be driven by control sequences of different origins ranging from tumour virus DNA to phage or plasmid DNA of microbial cells. It was considered that the origins of these control sequences do not influence their effectiveness in insertional activation of proto-oncogenes, but that their strength will do so. Because the cells containing the vector have their own strong regulatory sequences, the added

vector control elements have only a small additional effect. If, however, the vector sequences are amplified their relative importance may increase. This means that contaminating strong control sequences may increase the probability of cell transformation. It was also recognized that bacterial or yeast promoters are less effective in mammalian cells and vice versa. If control sequences derived from tumour viruses are used, these must be limited in length to ensure that they do not include sequences encoding for oncoproteins.

Although it is most unlikely that the presence of heterogeneous DNA in a vaccine (regardless of its origin) would induce a premalignant change, a system with the least chance of inducing cell transformation in vaccine recipients should preferably be used; the purity and efficacy of the vaccine should also be maintained because it could be used in many millions of young infants or newborns and the latent period before a neoplastic disease appears may be 10–30 years or longer. In addition, an increased risk, although not demonstrated, may exist in certain individuals and population groups owing to genetic abnormalities or because of inherited or acquired immune deficiencies, or other environmental influences.

While the acceptable amount of heterogeneous DNA in vaccine preparations cannot be defined accurately, it should be as low as is reasonably attainable. A maximum level of 100 pg per parenteral dose has been generally used in the past and provides a good margin of safety, based on experimental and theoretical grounds. This level was chosen because determinations of lower levels of DNA are not always precise and a level of less than 100 pg appears to be easily attainable (3).

Proteins

The participants at the meeting reaffirmed the conclusion that the presence of contaminating proteins, at the concentrations in which they are ordinarily found in appropriately purified vaccines, does not constitute a serious risk when prepared in continuous cell lines except when they act as immunogens.

Use of live viruses

Oral live attenuated virus vaccines

Contaminating heterogeneous DNA is thought to present an even lower risk in orally administered vaccines than in vaccines given parenterally because of the nucleases or other factors in the intestine. However, clear evidence for this assertion is not available. Therefore, removal of DNA from products intended for oral administration and prepared from continuous cell lines that are considered suitable as substrates for the preparation of injectable products is desirable. In

the case of replicating viruses, the choice of purification steps must take into account the possible effects on the properties of the vaccine virus.

Viruses used as vectors

In the production of live virus vaccines or the production of antigens in cell cultures herpes or vaccinia virus strains without neurovirulence, adenoviruses, attenuated poliovirus type 1, some insect viruses and possibly others are being used for the construction of hybrid viruses. Each case must be evaluated individually for efficacy and safety. The same applies to the use of certain bacteria with inserted viral genomes for use as orally administered live vaccines.

Selection of cell substrates

In the selection of cell substrates for vaccine production, the following prerequisites as adopted by one National Regulatory Authority (US Public Health Service, FDA) should be considered:

- available data on the cell substrate;
- potential risk;
- case-by-case decision with a risk-benefit assessment;
- purity of the final product;
- inactivation steps during manufacture;
- target population for the product; and
- alternative production systems with a potentially lower risk.

Conclusions

The formulation of policy and regulatory standards for vaccines and their safety is highly complex and must be based, in part at least, on speculation and informed judgement in the absence of definitive information. The participants emphasized the need for a continuous dialogue to review, develop, and maintain a meaningful consensus among a broad-based multidisciplinary section of the informed scientific community. They were satisfied that some continuous cell lines were a suitable substrate for the production of vaccines and indeed may, in the future, be the only available source of certain products, but their use must be considered on a case-by-case basis and be subjected to continuing surveillance.

References

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